(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 June 2004 (10.06.2004)

PCT

(10) International Publication Number
WO 2004/047880 A1

(51) International Patent Classification[7]: A61L 27/34, 27/32, 27/46, B05D 1/00

(21) International Application Number: PCT/IL2003/000975

(22) International Filing Date: 18 November 2003 (18.11.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/428,725 25 November 2002 (25.11.2002) US

(71) Applicants *(for all designated States except US)*: **YISSUM RESEARCH AND DEVELOPMENT OF THE HEBREW UNIVERSITY OF JERUSALEM** [IL/IL]; Hi-Tech Park, The Edmond J Safra Campus, Givat Ram, Jerusalem, POB 39135, 91390 Jerusalem (IL). **UNIVERSITE LOUIS PASTEUR** [FR/FR]; Institut le Bel, 4, rue Blaise Pascal, F-F-67000 Strasbourg (FR).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **MILHOFER-FUREDI, Helga** [IL/IL]; 13 HaNamer Street, 96954 Jerusalem (IL). **BAR YOSEF OFIR, Pasit** [IL/IL]; Givat Bet Hakerem 4, 96268 Jerusalem (IL). **SIKIRIN, Maja** [HR/IL]; 28 Zichron Tuvia Steet, 94314 Jerusalem (IL). **GERGELY, Csilla** [HU/FR]; 15 rue du Fourbourg de Saverne, F-67000 Strasbourg (FR). **CUISINIER, Frederic** [FR/FR]; 14 bassemerlusse St., F-88490 LUSSE (FR).

(74) Agent: **BRESSLER, Eyal**; Dr Eyal Bressler Ltd, 8 Hamarpe St., POB 45125 Har Hotzvim, 91450 Jerusalem (IL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/047880 A1

(54) Title: ORGANIC-INORGANIC NANOCOMPOSITE COATINGS FOR IMPLANT MATERIALS AND METHODS OF PREPARATION THEREOF

(57) Abstract: The present inventions provides for a novel organic-inorganic composition, comprising a plurality of organic polyelectrolytes films (SAPF), interspaced with a plurality of films of nanometer to micron-sized inorganic amorphous or crystalline bioactive particles. This sequentially adsorbed nanocomposite film is especially useful for coating implants. The present invention also provides a cost effective and efficient method of preparing calcium phosphate embedded organic polyelectrolytes compositions. The method comprising inter alia the steps of adsorbing polyelectrolytes on top of a surface so that at least one electrolyte film is obtained; and depositing calcium-containing compositions on top of said polyelectrolyte multilayer film, so that at least one nanometer to micron-sized layer comprising calcium phosphate is formed; so a calcified SAPF is obtained.

ORGANIC-INORGANIC NANOCOMPOSITE COATINGS FOR IMPLANT MATERIALS AND METHODS OF PREPARATION THEREOF

FIELD OF THE INVENTION

The invention generally relates to organic-inorganic composite coatings for implant materials, mainly referring to orthopedic and dental implants, and to methods of preparation thereof.

BACKGROUND OF THE INVENTION

While most metals and metal alloys meet many of the biomechanical requirements of load bearing implants, they are bioinert or biotolerant and thus show poor or nonexistent interfacial bonding between the metallic surface and the surrounding bone. To alleviate this problem, different surface coatings consisting of calcium phosphates have been applied. Coating methods previously employed with some success include plasma spraying, which gives tight adhesion between hydroxyapatite and the metal plate. Drawbacks of this method are that it requires costly equipment and high processing temperatures. The high temperatures employed cause significant structural alterations in the coatings, which may result in mechanical failure at the interface metal-coating interface and within the coating itself.

More recently processes for obtaining hydroxyapatite coatings by direct precipitation onto the implant material from solutions containing calcium and phosphate ions and/or various foreign ions (including magnesium, carbonate, or other) have been proposed. In US Pat. No. 5,188,670 assigned to Brent, a complicated process and apparatus for coating porous substrates with hydroxyapatite film has been described. Essentially, the method comprises combining calcium and phosphate solutions of relatively high concentrations, at elevated temperatures between 60 to 90°C, to obtain hydroxyapatite crystals, which are then, in a specially designed apparatus, precipitated onto the surface to be coated. Coating methods disclosed in US Pat. No. 6,280,789 assigned to Rey et al., and further in US Pat. No. 6,207,218, assigned to Layrolle et al. are simpler, in both procedures the material to be coated, e.g., a medical implant was

submerged in an aqueous solution containing calcium, phosphate and bicarbonate ions and spontaneous precipitation of carbonated apatite was initiated in the presence of the implant by raising the supersaturation *in situ*. The supersaturation was regulated by either raising the temperature, and thus the pH, by removing some of the carbonate or by bubbling alternately CO_2 or air through the solution. A drawback of these methods is that the coatings are simply precipitated onto the substrate surface but are in no way anchored to it. They are thus likely to be unstable and not likely to withstand rough implanting procedures. A related procedure, described in the art is based on soaking a metal substrate for two weeks in very dilute solutions, containing calcium, phosphate and other inorganic ions, which would produce a calcium phosphate coating. Optionally one or more biologically active, organic substances could be co-precipitated. This method seems to suffer from the same problems as above, which the authors were trying to overcome by adjusting the surface roughness of the substrate and using prolonged coating times, thus inducing slow growth from very dilute solutions. Consequently, the method is rather time consuming and the deposits are ill defined in terms of composition and structure. The coatings showed cracking and fractures and their appearance was dependent on both the material and the surface of the metal substrates used.

A new approach of producing calcium phosphate coatings, presented by Bunker et al., *Science* **264,** 1994, 48, calls for modifying substrate surfaces by introducing functional groups, which should mediate the deposition of calcium phosphate mineral under mild conditions. The idea is based on the observation that in nature organisms use various macromolecules, containing different functional groups, i.e. carboxylic, sulfate and phosphate groups, to induce and control mineralization. Accordingly, it was assumed, that on functionalized surfaces mineralization would readily proceed from relatively dilute solutions at low temperatures and under mild conditions (close to physiological). Such methods should be cost-effective and adaptable to a variety of ceramic, polymeric and metallic materials. Various methods to introduce functional groups into different substrates have been proposed.

Many investigators, such as Kokubo and collaborators (See *Acta Mater.* **46,** 1998, 2519; Materials Science Forum **293,** 1999, 65 for example) tried to introduce functional groups to various substrates, such as bioglass, glass ceramics and titanium metal surfaces. The methods of treatment depended on the specific substrate, to which

coating was to be applied. Titanium plates where soaked for 24h in concentrated NaOH solutions and subsequently heated to a temperature between 500 to 600°C. Coatings were then deposited by soaking the plates for several days in a so-called simulated body fluid, SBF, i.e. a solution of ionic concentrations similar to those in blood plasma. Samples thus treated showed relatively high bonding strengths, in comparison to bioglass and glass ceramics, between the coating and the metal surface. It was further shown that titanium plates functionalized with Ti-OH and Ti-OOH groups specifically induce oriented crystallization of hydroxyapatite and octacalcium phosphate (OCP).

However, the coatings described were not well defined in terms of composition and structure and were nor evenly spread over the coated surface. Also, the proposed methods are rather time and energy consuming.

To enhance the speed of deposition and the thickness of the coatings, the inventors of US Pat. No. 6,129,928 to Sarangapani *et al.* proposed to covalently bind a nucleating agent with acidic functional groups to the surface hydroxyl groups of titanium plates. In addition, post-treatment with diluted hydrogels is proposed, to reinforce the inorganic structure and enhance the mechanical strength of the coating. Growth factors and other reactive proteins can be included, by coupling them to the hydrogel molecules. Although this patent presents a significant improvement over previous art, the method is substrate specific, as it presupposes a substrate with reactive surface hydroxyl groups, to which a nucleating agent can be covalently bound.

Finally, US Pat. No. 2002/018798 to Dard et al. discloses coatings, comprising an organic-inorganic composite system, which consists of a collagen matrix mineralized with calcium phosphate. The collagen matrix is prepared by immersing the substrate into a solution of collagen type I, which is then reconstituted by adjusting the pH and temperature. The collagen fibrils thus obtained are mineralized by an electrochemical method, in which the coated substrate serves as one of the electrodes. Thus, since the substrate has to be conductive, the method is restricted to metals. Also, although the material is similar to bone tissue, it does not contain acidic functional groups, which are thought to be responsible for biological mineralization.

Most recently, US Pat. No. 2002/0037383, assigned to Spillman et al. disclosed a method to enhance the biocompatibility of medical devices by introducing

electrostatically self-assembled thin film coatings. No calcium phosphate mineral was included into such coatings.

The present invention is based on experience known in the art with polyelectrolyte multilayer films, as well as with the crystallization of calcium phosphates and their interactions in solution with polyelectrolytes and extracellular matrix proteins. It has been shown in the art that it is possible to fabricate polyelectrolyte multilayer films on substrates by consecutive adsorption of polyanions and polycations or other charged molecular or colloidal objects. Such films are mainly dependent on the properties of the chosen polyelectrolytes and much less on the underlying substrate or the substrate charge density. It has also been demonstrated in the art that nucleation and growth of calcium phosphate crystals in aqueous solutions is induced by an amorphous precursors phase, and the crystal morphology is specifically influenced by polyelectrolytes, such as polyaminoacids and matrix proteins, which may be present in solution. A method for changing the surface free energy, based on multilayer film, was shown to increase the nucleation activity of surfaces. In order to combine the high nucleation activity of calcium phosphate crystals and of polyelectrolyte and thus enhance the bioactivity of orthopedic and dental implants, we have developed methods for embedding calcium phosphate, adsorbed and/or grown *"in situ"* on polyelectrolyte multilayer films.

SUMMARY OF THE INVENTION

It is thus an object of the present invention to provide an organic-inorganic composition, comprising a plurality of organic polyelectrolyte films, interspersed with a plurality of films of nanometer to micron-sized inorganic amorphous or crystalline bioactive particles; so that a sequentially adsorbed nanocomposite film is obtained. The aforementioned polyelectrolytes are preferably selected from the group of polyaminoacids, poly-ariginine acid, poly-leucine, poly-arginine, poly-lysine, poly-glutamic acid, poly-serine, poly-aspartic, poly-hydroxyproline, poly(lactide), polyinosinic acid, polycytidylic acid, polythymidilic acid, polyguanylic, poly(styrene), poly(ethylene), poly(oxyethylene), poly(acrylic) acid, poly(methacrylic) acid, poly (ethylene glycol), poly(galacturonic) acid, poly(maleimide), silk, amelogenin, albumin, sialoprotein, osteocalcin,

phosphophoryn, phosvitin, polysaccharides, polyphosphonates, polyphosphates, phosphoproteins, lectines, lipopolysacharide, fibrinogen, fibronectin, heparin, lactic acid, glycolic acid, dextrin, cyclodextrin, or any bioactive polymers, such as chitosan, hyaluronic acid, agarose, alginate, collagen, glucosaminoglycan, heparan, chondroitin, chondroitin sulfate, glycin, elastin, cellulose, proteoglycan, their derivatives or any mixture thereof. The hereto-defined bioactive inorganic layers preferably comprise crystalline calcium phosphates. More specifically, the aforementioned crystalline calcium phosphates comprise calcium hydrogen phosphate, octacalcium phosphate, tri-calcium phosphate, calcium deficient apatite, carbonated apatite, stoichiometric hydroxyapatite, crystalline calcium phosphates containing foreign ions, crystalline calcium phosphates containing cytokines, crystalline calcium phosphates containing peptides, their derivatives or any combination thereof. Additionally or alternatively, the hereto-defined bioactive inorganic layers comprise bioactive glass, amorphous calcium phosphate (ACP) or any combination thereof.

It is also in the scope of the present invention to provide a most effective bioactive nanocomposite coating comprising the composition as defined in any of the above. Moreover, it is further in the scope of the present invention to provide implants, comprising the aforementioned compositions. More specifically, hereto-defined implants are at least partially coated by the aforementioned compositions, in the manner that a significant portion of said implants are coated by a bioactive nanocomposite. Those implants are preferably comprised of materials selected from composite materials, glass ceramics, polymer, metal, metal alloys, or any combination thereof. The said metal or metal alloy are at least partially made of titanium, titanium based alloys, stainless steel, tantalum, zirconium, nickel, tantalum, iridium, nobium, palladium, nickel-titanium, alloys based thereon or any combination thereof.

It is another objective of the present invention to provide a simple method for preparing calcium phosphate embedded organic polyelectrolyte compositions. This method is basically comprised of two or more of the following steps: (a) adsorbing polyelectrolytes on top of a surface so that at least one polyelectrolyte film is obtained; and then (b) depositing calcium containing compositions on top of said polyelectrolyte multilayer film, so at least one of nanometer to micron-sized layer comprising calcium phosphate is formed, so that a calcified SAPF is obtained.

It is hence in the scope of the present invention to provide a method comprising *inter alia* the steps of adsorbing polyelectrolytes on top of a surface so that at least one polyelectrolyte film is obtained; washing the obtained film in the manner that residual polyelectrolytes are removed; depositing nano-sized to micron-sized particles of bioactive glass, amorphous calcium phosphate and/or crystalline calcium phosphate particles on top of the obtained polyelectrolyte multilayer film, so that at least one layer comprising calcium-containing bioactive inorganic material is obtained; and then washing the obtained calcified film in the manner that residual calcium containing solution is removed; wherein an SAPF which is at least partially covered by a single inorganic layer is obtained.

It is also in the scope of the present invention to provide a method comprising *inter alia* the steps of adsorbing polyelectrolytes on top of a surface so that at least one polyelectrolyte film is obtained; washing the obtained film in the manner that residual polyelectrolytes are removed; depositing nano-sized to micron-sized particles of bioactive glass, amorphous calcium phosphate and/or crystalline calcium phosphate particles on top of said film, so that at least one layer comprising calcium-containing, bioactive inorganic material is obtained; washing the obtained calcified film in the manner that residual calcium containing solution is removed; and, adsorbing polyelectrolytes on top of said calcium phosphate layer; wherein said sequence of steps is repeated in the manner that calcified SAPF is obtained.

It is further in the scope of the present invention to provide a method comprising *inter alia* the steps of adsorbing polyelectrolytes on top of a surface so that at least one film is obtained; washing the obtained film in the manner that residual polyelectrolytes are removed; depositing nano-sized to micron-sized particles of bioactive glass and/or ACP on top of said SAPF, so that at least one film comprising calcium-containing, bioactive inorganic material is formed; washing the obtained film in the manner that residual calcium containing solution is removed; and then immersing the material into a calcifying solution in the manner that the growth of crystalline calcium phosphate is induced and sustained.

It is lastly in the scope of the present invention to provide a method comprising *inter alia* the steps of adsorbing polyelectrolytes on top of a surface so that at least one film

is obtained; washing the obtained film in the manner that residual polyelectrolytes are removed; depositing nano-sized to micron-sized particles of bioactive glass, and/or ACP on top of said SAPF, so that at least one film comprising calcium-containing bioactive inorganic material is formed; washing the obtained calcified film in the manner that residual calcium containing solution is removed; adsorbing polyelectrolytes on top of said calcium phosphate layer; wherein said sequence of steps is repeated in the manner that calcified SAPF is obtained; and then immersing the obtained material into a calcifying solution in the manner that *in situ* growth of calcium phosphate crystals is induced and sustained within the calcified SAPF.

BRIEF DESCRIPTION OF THE INVENTION

In order to understand the invention and to see how it may be implemented in practice, a plurality of preferred embodiments will now be described, by way of non-limiting example only, with reference to the accompanying figures, in which

Fig. 1A, 1B and 1C are representing data recorded by the OWLS technique for the build-up of SAPF, $(PLL/PGA)_5PLL$ (a); $(PLL/PGA)_6$ (b) and (c) from MES/TRIS buffer (a) and (b) and from HEPES buffer (c), and the adsorption of ACP from water [(a) and (b)] and from HEPES buffer (c), respectively;

Fig. 2 is representing SEM micrographs (two different magnifications) of aggregated ACP particles deposited on glass, coated with $(PLL/PGA)_{15}$

Figs. 3 and 4 are showing SEM micrographs of ACP particles in coatings A (Fig.3, two magnifications) and coatings A and B (Fig. 4, side view) respectively;

Fig. 5 is showing SEM micrographs of coating C, two different magnifications;

Fig. 6 is showing SEM micrographs of: (a) coating C and (b) coating D, side views;

Fig.7 is showing surface morphologies of coatings C (a-b) and D (c-f) before and after the adhesive tape test;

Fig. 8 is showing adhesion and proliferation of human osteoblast cells onto bare titanium (L1), OCP deposited on bare titanium (L2), titanium coated with $(PLL/PGA)_{10}$ (L3), titanium coated with $(PLL/PGA)_{10}$-OCP-$(PLL/PGA)_5$

(coating C; L5), titanium coated with $(PLL/PGA)_{10}$-OCP-$(PLL/PGA)_5$-OCP-$(PLL/PGA)_5$ (coating D; L7) and plastic (L8); and,

Fig. 9 is illustrating the adhesion and proliferation of human osteoblast cells seeded onto PE multilayer film and nanocomposites $(PLL/PGA)_3$PLL-HA $(PGA/PLL)_3$ and $(PLL/PGA)_2$ PLL-HA-$(PGA/PLL)_2$ –HA-$(PGA/PLL)_2$.

DETAILED DESCRIPTION OF THE INVENTION

The following description is provided, alongside all chapters of the present invention, so as to enable any person skilled in the art to make use of said invention and sets forth the best modes contemplated by the inventors of carrying out this invention. Various modifications, however, will remain apparent to those skilled in the art, since the generic principles of the present invention have been defined specifically to provide bioactive organic-inorganic nanocomposite coatings for implant materials and to methods of preparation thereof.

The present invention generally relates to organic-inorganic coatings, comprising a sequentially adsorbed polyelectrolyte films (i.e., SAPF), interspersed with layers of nanometer to micrometer sized amorphous, calcium phosphate particles, calcium phosphate crystals and/or bioactive glasses. The SAPFs are constructed as previously described by Decher, G. *Science* 277 (1997) 1232, by consecutively adsorbing positively and/or negatively charged polyions from their respective solutions.

For the purpose of the present invention, organic polyelectrolytes are selected in a non-limiting manner from biocompatible or at least partially biocompatible polyelectrolytes, such as polyaminoacids, polysaccharides, polyphosphonates, polyphosphates, phosphoproteins, and any other synthetic or natural biocompatible or partially biocompatible polymers and/or mixtures of the same etc., all hereto denoted in the term organic 'PE'.

Hence, it is in the scope of the present invention wherein a plurality of polycation compositions is sequentially adsorbed on top of a plurality of polyanion PE films or *vice versa*. It is further in the scope of the present invention wherein polycation or

polyanion composition is sequentially adsorbed on top of another polycation or polyanion layer, respectively. It is still in the scope of the present invention wherein nonionic compositions are utilized *inter alia* in said sequentially adsorbed PE films.

Further according to the present invention, the term 'SAPF' is referring to any film comprising sequentially adsorbed PE films, e.g., a multilayer matrix or a multi-stratum matrix, a conglomerated matrix, a crystallized matrix, amorphous structures, vesicular or sponge like structures or any combination thereof.

Moreover, the term 'film' generally relates according to the present invention to any homogeneous or heterogeneous, continuous or discontinuous, isotropic or anisotropic bioactive films, coatings or layers, at least partially comprising SAPF as defined in any of the above.

The term 'bioactive' is generally referring to bioactive calcium-containing compositions, composites and devices. It is acknowledged in this respect that bioinert portions provided in those compositions are also possible. The materials according to the present invention can also be biodegradable in the manner that it is either dissolved or resorbed in the body. It is according to yet another embodiment of the present invention, wherein the term 'bioactive' is also referring to any at least partially biocompatible compositions, composites and devices.

Alternating with SAPF are layers of nanometer to micrometer sized calcium phosphate particles, or other inorganic particles, such as bioactive glasses, adsorbed and/or embedded within the PE multilayer matrix. It is well in the scope of the present invention wherein the aforementioned phosphate particles, or other inorganic particles have inorganic polyelectrolyte characteristics. It is also in the scope of the present invention wherein the inorganic bioactive particle layers comprise bioactive glasses, amorphous calcium phosphate, and/or crystalline calcium phosphates, such as calcium hydrogenphosphate, octacalcium phosphate, tricalcium phosphate, calcium deficient apatite, carbonated apatite, stoichiometric hydroxyapatite with specific properties or mixtures of some of the above. It is yet acknowledged in this respect that other inorganic bioactive layers of different compositions are possible.

The term 'bioactive glasses' is generally referring according to the present invention to any calcium containing bioactive glasses, such as SiO_2-Na_2O/K_2O-CaO/MgO-

B_2O_3-P_2O_5 matrices that will give, after immersion in simulated body fluid and/or calcifying solution, a bioactive surface and/or layer.

According to one embodiment of the present invention, the calcium phosphate is grown directly on and/or in the film during the building up period. Hence, the preparation of calcium phosphate layers is based on the adsorption or embedding of amorphous calcium phosphate particles, hereto defined in the term 'ACP' and/or bioactive glass, into the SAPF and subsequent growth of crystalline octacalcium phosphate or calcium deficient hydroxyapatite from a metastable supersaturated solution, henceforth calcifying solution, crystal growth being induced and mediated by the ACP or bioactive glass particles and/or the polyelectrolyte constituting the top layer. Thus the SAPF - calcium phosphate assembly is formed by the following sequence of steps:

i. adsorbing a sufficient amount of organic PE onto a predetermined substrate surface;
ii. cleansing said upper layer of said substrate at least partially coated by said organic composition by means of removing the residual polyelectrolyte(s) by washing;
iii. depositing ACP and/or bioactive glasses or any mixture thereof from a suspension on the top layer of said cleansed organic PE film, so that at least one nanometer to micron-sized layer comprising calcium containing matrix is obtained;
iv. removing the residual calcium containing solution by washing;
v. adsorbing polyelectrolytes on top of said calcium phosphate and/or bioactive glass layer; and,
vi. optionally repeating said procedure until a SAPF comprising a plurality of N organic PE films alternating with M layers of inorganic particles is formed, wherein $N \geq 1$ and $M \geq 1$.

The obtained SAPF is then immersed into a calcifying solution for a specified time, until the desired crystalline precipitate is formed. The calcifying solution comprises a solution containing calcium and phosphate ions and/or any other ions in an effective amount necessary for a particular purpose. Said solution is supersaturated but

metastable, meaning that no precipitate should form without the presence of a "seeding" substrate.

After the desired crystalline calcium phosphate layers have been formed, the residual calcium phosphate solution is removed by washing and optionally; the coated samples are dried and prepared for further use.

Moreover, it is according to yet another preferred embodiment of the present invention to provide methods wherein calcium phosphate crystals or glass ceramic particles are synthesized according to specifications and then embedded into the SAPF. By this procedure PE multilayers alternating with nano – or micron-sized calcium phosphate crystals containing trace metal ions or particles having bioactive properties can be prepared.

Coatings, prepared according to one or more of the aforementioned methods are either transferred to any suitable substrate, preferably to substrates at least partially made of materials selected from composite materials, glass ceramics, polymer, metal, and metal alloys, and/or built directly on top of the surfaces. A suitable metal will be chosen from the group of bioinert metals or metal alloys, which are deemed suitable for metal implants with load-bearing applications. Such are titanium, titanium based alloys, (Ti-6Al-4V and others), stainless steel, tantalum, zirconium, alloys based thereon, etc.

It is in the scope of the present invention wherein the aforementioned compositions are forming, coating, filling, replacing or reinforcing implants. It is also in the scope of the present invention wherein the aforementioned term 'implant' is denoted in a non limiting manner for any biodegradable or nondegradable implants; prosthetic components; bone substitute materials, artificial bone materials, glues, sealants or cements; orthopedic or other surgical inserts; dental implants, dental prosthesis or any combination thereof. It is also in the scope of the present invention wherein said implant is characterized by any suitable shape or size in the manner that it is adapted to be inserted into or onto humans or animals body.

It is also in the scope of the present invention wherein the said implants provided according to the present invention can be used for drug delivery, controlled release or sustained release of minerals or salts; organic substances; medicaments; drugs;

cytokines, hormones, regulators of the bone metabolism and growth; antibiotics, biocide and bactericide drugs or peptides, DNA, RNA, amino acids, peptides, proteins, enzymes, cells, viruses and/or a combination thereof.

EXAMPLE 1

Buildup of SAPF and adsorption of amorphous calcium phosphate on glass plates.

Materials and methods: Poly(L-lysine) (PLL, MW 3.26 x 10^4 Da), poly(L-glutamic acid) (PGA, MW 7.2 x 10^4 Da), tris(hydroxymethyl) aminomethane (TRIS), 2-(*N*-morpholino) ethanesulfonic acid (MES), N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) and NaCl from Sigma and ultrapure water, UPW (Milli Q-plus system, Millipore or Barnstead) were used. MES/TRIS/NaCl or HEPES buffer solutions of pH 7.4 were prepared as follows: MES/TRIS/NaCl buffer: 25 mmol of MES, 25 mmol of TRIS and 100 mmol of NaCl were dissolved in 1 liter of UPW. HEPES/NaCl buffer: 25 mmol of HEPES and 150 mmol NaCl were dissolved in 1 l of UPW. Polyelectrolyte solutions were always freshly prepared by direct dissolution of the respective adequate weights in filtered buffer solutions. Suspensions of ACP were freshly prepared for each experiment by rapidly mixing equal volumes of 3, 5 or 10 mmolar equimolar solutions of calcium chloride and sodium phosphate in UPW or in HEPES buffer. The sodium phosphate solutions were adjusted to pH 7.4 before mixing.

The deposition of $(PLL/PGA)_i$ (wherein i is the number of layer pairs) and subsequent deposition of ACP was demonstrated by Optical Waveguide Lightmode Spectroscopy, denoted hereto in the term 'OWLS', and/or visualized by scanning electron microscopy, denoted hereto in the term 'SEM'.

The optical waveguide lightmode spectroscopy technique (i.e., OWLS) is an optical technique, which gives information on the quantity, thickness and effective refractive index of an adsorbed layer onto a planar waveguide. OWLS is based on the effective refractive index change of a waveguide during the adsorption processes. Laser light, which is incoupled into the waveguide, is recorded and is proportional to the adsorbed amount of material. PLL/PGA PE films were built *in-situ* in the OWLS cell. In order to perform measurements, the system was rinsed with buffer, to remove all

impurities. After the buffer flow was stopped, 100 μL of poly-L-lysine solution were manually injected into the cell through the injection port. After 12-15 min, sufficient to reach a plateau, the buffer flow was restarted for 12-15 min to rinse the excess material from the cell. In the same way the alternate adsorption of polyanions and polycations was continued and, progressively, $(PLL/PGA)_i$ multilayers were deposited. The film build-up was stopped for $i = 6$ to obtain a negatively charged surface and for $i = 5$ plus PLL to obtain a positive surface. After completion of the respective multilayer, 300 µl of a freshly prepared suspension of ACP were injected several times. Before the addition of ACP the system was rinsed for about 15 min with UPW, adjusted to pH 7.4 (Figs. 1a,b) or HEPES buffer, pH 7.4 (See Fig. 1C).

For SEM (JOEL JSM-840 Scanning Microscope) analysis samples were prepared separately on glass plates, wherein the procedure was the same as in the OWLS experiment (see above). After deposition of ACP all plates were washed with UPW. dried in a stream of nitrogen and kept at 4°C until analysis.

Reference is made now to Fig. 1A and Fig. 1B, showing the data recorded by OWLS for the build-up of SAPF from MES/TRIS buffer, ending with a positive (a) and a negative (b) film respectively. Also shown is the subsequent adsorption of ACP particles thereon. The continuous increase of the refractive index in the transverse electric mode, N(TE), shows the alternate deposition of the polyelectrolytes. One can observe the step by step layering of the polyelectrolyte films, each time followed by a plateau during the rinsing step. Rinsing of the SAPF with UPW before introducing the ACP suspension causes a slight decrease of the refractive index, followed by an increase, indicating the adsorption of ACP particles. Fig. 1c shows the build-up of $(PLL/PGA)_6$ from HEPES buffer and the subsequent deposition of ACP particles. No decrease in the refractive index is apparent because there was no change in the medium before and during the introduction of ACP.

Reference is made now to Fig. 2, presenting SEM micrographs of aggregated ACP particles deposited on glass plates, coated with $(PLL/PGA)_{15}$ SAPF (two different magnifications). Similar SEM micrographs were obtained when ACP was deposited on $(PLL/PGA)_{14}PLL$. It is obvious from the above results, that ACP could be adsorbed on both positively and negatively charged multilayer films.

EXAMPLE 2

Coatings A and B - build-up of SAPF on Ti plates and deposition of ACP particles upon them.

Materials and Methods: Pure titanium plates, were received courtesy of Dentaurum, J.P. Winkelstroeter AG, Germany (Titanium ASTM grade 4, diameter 15 mm, thickness 1.5 mm, machine polished to a surface roughness Ra 0.4 μm, Rmax 3.0 μm and cleaned in perchloroethylene) and courtesy of SAMO S.p.A., Italy (Titanium ASTM grade 2, 1 x 1 cm, thickness 1.5 mm, chemically etched by SAMO). Before coating, plates were sonicated subsequently in acetone (p.a.), ethanol (p.a.) and three times in UPW. Each procedure lasted 10 - 15 min. XRD spectra of the bare plates showed only peaks characteristic of Ti.

Coating A: $(PLL/PGA)_i$ and $(PLL/PGA)_iPLL$ ($i = 9$ or 14) multilayers were deposited as described in Example 1, using 1 ml of the respective solutions of PLL, PGA and HEPES/NaCl buffer pH 7.4. The plates with adsorbed multilayers were washed with buffer before depositing ACP particles. Plates were dipped three times into suspensions of ACP prepared in HEPES buffer as described in example 1, using 10 mmolar equimolar solutions of calcium chloride and sodium phosphate. After deposition the ACP plates were washed with buffer.

Coating B: Coatings were prepared by depositing $[(PLL/PGA)_5\text{-ACP}]_i$ or $[(PLL/PGA)_4PLL\text{-ACP}]_i$ (i = 1-4) on coatings A. The preparation could be demonstrated by OWLS (not shown).

After preparation of coatings A and B all plates were washed with buffer, dried in a stream of nitrogen and kept at 4°C until further analysis. Samples thus prepared were observed by scanning electron microscopy (JOEL JSM-840 Scanning Microscope) and analyzed by powder X-ray diffraction.

Reference is made now to Figs. 3 and 4 showing four scanning electron micrographs of aggregated ACP particles in coatings A and B. As expected, surface coverage is denser in coating B. XRD diffraction patterns showed only Ti peaks, indicating that the deposited calcium phosphate phase is indeed amorphous.

EXAMPLE 3

Coatings C and D obtained by build-up of SAPF on Ti plates and in-situ growth of OCP crystals.

Coatings C and D: Coatings A and B, respectively, were prepared on Ti plates as described in Example 2. Thus prepared plates were immersed into a calcifying solution (2.8 mmol / l $CaCl_2$, 2 mmol / l Na_2HPO_4, 25 mmol / l HEPES, 150 mmol / l NaCl, pH 7.4) for 48 hours. By this procedure coating A converted into coating C, whereas coating B gave coating D. After the crystallizing procedure all plates were washed with buffer, dried in a stream of nitrogen and kept at 4°C until further analysis by X-ray powder diffraction and SEM. The adhesive tape test was conducted according to ASTM D 3359-92a and the tested specimens were observed with SEM.

Reference is made now to Fig. 5 showing SEM micrographs of coating C. Large, well developed, spherically oriented plate-like crystals were obtained. Apparently, the crystals grew from the previously deposited aggregated ACP particles (see Fig. 3B, Example 2).

Reference is made now to Fig. 6, presenting side views of SEM micrographs of: (a) coating C and (b) coating D. As in Example 2., the surface coverage improved with the number of SAPF's and ACP deposition steps, i.e. surface coverage of the plates was better in the case of coating D as compared to coating C.

Reference is made now to Fig. 7, presenting the results of the adhesive tape test, showing that most of the coatings (including the crystals, Figs. 7 e, f) remained intact on the Ti plates, indicating that the bonding between the plates and coatings C and D is good.

Reference is made now to Fig. 8, presenting a cell culture experiment. The cells were human primary osteoblast and were deposited onto six different substrates. Three substrates, L1, L2 and L8, respectively, are the reference standards and the golden standard for osteoblast cell adhesion and proliferation. The cell proliferation obtained after 14 days proved the bioactivity of organic-inorganic nanocomposites C (L5) and D (L7) as compared to bare titanium (L1) and titanium coated only by SAPF (L3), or inorganic particles (L2).

EXAMPLE 4

Coatings E obtained by build-up of SAPF containing micron-sized bioactive glasses and *in situ* growth of apatite crystals.

Organic-inorganic nanocomposite was prepared essentially as described in Example 3, wherein micron-sized bioactive glass particles, commercially available **from** Mo-Sci Corp., which are especially adapted for hard/soft tissue bonding, were used instead of ACP particles. This glass composition is about 45% SiO_2, 24.5% Na_2O, 24.5% CaO and 6% P_2O_5 and is generally characterized by specific gravity of 2.7 g/cm^3; refractive index (n_D) 1.55; softening temp. (Ts) of about 550°C; dissolution rate nearly 150 $\mu g/cm^2/day$; elastic modulus ranges between 30 to 35 GPa; tensile strength ranges between 40 to 60 MPa; and thermal exp of about 1.6×10^{-7} cm/°C. SEM and OWLS presentations are not presented.

EXAMPLE 5

Human osteoblast cell adhesion and proliferation onto PE multilayer films containing synthesized hydroxyapatite crystals

Materials and Methods: SAPF was prepared essentially as described in Examples 1 and 2, but to allow for better cell growth, 25 mM N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] buffer (HEPES, pH 7.4) in 137 mM NaCl solution was used as medium, to construct the PE multilayers. Organic-inorganic nanocomposite films were then constructed by adsorbing previously prepared hydroxyapatite crystals (HA, synthesized in the laboratory) within the PE multilayers. The composition of the nanocomposite films was as follows:

Nanocomposite 1: $(PLL/PGA)_3$ PLL-HA-$(PGA/PLL)_3$

Nanocomposite 2: $(PLL/PGA)_2$ PLL-HA-$(PGA/PLL)_2$-HA-$(PGA/PLL)_2$.

Human osteoblast cells were seeded onto nanocomposites 1 and 2 and onto a PE multilayer film: $(PLL/PGA)_6$ PLL which was used as control.

Reference is made now to Fig. 9, illustrating the adhesion and proliferation of human osteoblast cells seeded onto SAPF and nanocomposites 1 and 2. Fig. 9 convincingly shows the increased adhesion (day 1) and proliferation (days 3 and 7) of human

osteoblast cells in the nanocomposite systems, as compared to the pure PE multilayer film. It is also obvious, that the cell response increased with increasing number of the embedded inorganic layers (nanocomposite 1 vs. nanocomposite 2). This shows, that due to their porosity, the coatings are easily penetrated by human osteoblast cells. This property is essential for the induction of new bone formation and intergrowth of the implants within the bone tissue.

It is acknowledged in this respect that a plurality of methods for the construction of SAPF materials is applicable. The methods to deposit PE layers and inorganic particles are thus not restricted to injection of solutions onto the substrate, i.e., injection coating, but include also spraying and dipping methods. The surface to be coated can be any surface as defined above. Sequentially depositing on a surface alternating layers of polyelectrolytes may be accomplished in a number of ways. The depositing process generally involves coating and rinsing steps. One coating process involves solely dip-coating and dip-rinsing steps. Another coating process involves solely spray-coating and spray-rinsing steps. However, a number of alternatives, involving various combinations of spray-, dip-, injection-coating and/or rinsing steps, may be designed by a person having ordinary skills in the art. According to one preferred embodiment of the present invention, the aforementioned method is provided by means of depositing the PE calcified films.

Moreover, and according to yet another embodiment of the present invention, *in situ* growing films of crystalline calcium phosphate phases onto biocompatible SAPF have been provided. The nature of the calcium phosphate layers, grown *in situ* on the multilayer are strictly controlled, e.g., by controlling the experimental conditions and the time of exposure of the coated substrate to the calcifying solution. Any of the following mineral phases, octacalcium phosphate, calcium deficient apatite, carbonate apatite, hydroxyapatite or mixtures thereof may grow *in situ* under mild, close to physiological experimental conditions, e.g., low reactant concentrations, room temperature, approx. neutral pH etc. The present invention also provided to produce alternating layers, containing different calcium phosphate phases within the multilayer or embedding previously prepared mineral with specially designed characteristics.

Figs. 5 and 6 show that the calcium phosphate films, grown as described in the present invention, are thin, uniform, and porous characterized by a relatively large

surface area. The sizes of the crystals shown in Fig. 5b were between 1 and 2 μm, not exceeding 2 μm. By growing such crystalline films, a multilayered nanocomposite coating, consisting of alternating polyelectrolyte and calcium phosphate layers may be constructed as shown in example 3 (coating D). The resulting coating may be of any desired thickness and therefore should have the necessary strength and toughness, but also the porosity necessary for bioactive bone implants. By intergrowth (Figs 6A and 6B) with the SAPF the calcium phosphate layer fixed the nanocomposite coating to the underlying surface, so that very good adhesion was obtained (see Fig. 7).

The deposition and/or embedding of various synthetic calcium phosphate particles has been realized. In example 5 the influence on cellular activity of nanocomposite coatings, consisting of PE films alternating with previously synthesized hydroxyapatite has been demonstrated. It was clearly shown, that human osteoblast adhesion and proliferation is increased on organic-inorganic nanocomposites as compared to polyelectrolyte films and that cell activity increases with the number of inorganic layers.

Finally, in the examples given above, the adsorption of coatings proposed in this invention on two different substrates: glass, and metal, e.g., titanium, was demonstrated. In fact, such coatings can be deposited on any hydrophilic substrate regardless of size, shape and topology. The methods employed to produce the coatings are environmental friendly, cost effective, energy saving and simple to perform.

CLAIMS

1. An organic-inorganic composition, comprising a plurality of organic polyelectrolyte films, interspersed with a plurality of films of nanometer to micron-sized inorganic amorphous or crystalline bioactive particles; so that a sequentially adsorbed nanocomposite film is obtained.

2. The composition according to claim 1, wherein the polyelectrolytes are selected from the group of polyaminoacids, poly-leucine, poly-arginine, poly-lysine, poly-glutamic acid, poly-serine, poly-aspartic, poly-hydroxyproline, poly(lactide), polyinosinic acid, polycytidylic acid, polythymidilic acid, polyguanylic, poly(styrene), poly(ethylene), poly(oxyethylene), poly(acrylic) acid, poly(methacrylic) acid, poly(ethylene glycol), poly(galacturonic) acid, poly(maleimide), silk, amelogenin, albumin, sialoprotein, osteocalcin, phosphophoryn, phosvitin, polyphosphonates, polyphosphates, phosphoproteins, lectines, polysaccharides, lipopolysacharide, fibrinogen, fibronectin, heparin, lactic acid, glycolic acid, dextrin, cyclodextrin, or any bioactive polymers, such as chitosan, hyaluronic acid, agarose, alginate, collagen, glucosaminoglycan, heparan, chondroitin, chondroitin sulfate, glycin, elastin, cellulose, proteoglycan, their derivatives or any mixture thereof.

3. The composition according to claim 1, wherein the bioactive inorganic layers comprise crystalline calcium phosphates.

4. The composition according to claim 3, wherein the crystalline calcium phosphates comprise calcium hydrogen phosphate, octacalcium phosphate, tri-calcium phosphate, calcium deficient apatite, carbonated apatite, stoichiometric hydroxyapatite, crystalline calcium phosphates containing foreign ions, crystalline calcium phosphates containing cytokines, crystalline calcium phosphates containing peptides, their derivatives or any combination thereof.

5. The composition according to claim 1, wherein the bioactive inorganic layers comprise bioactive glass, amorphous calcium phosphate or any combination thereof.

6. Bioactive nanocomposite coatings comprising the composition as defined in claim 1 or in any of its depended claims.

7. Implants, comprising compositions as defined in claim 1 or in any of its dependent claims.

8. Implants at least partially coated by the compositions as defined in claim 1 or in any of its dependent claims in the manner that a significant portion of said implants are coated by a bioactive nanocomposite.

9. The implant according to claims 7 or 8; said implants are at least partially made of materials selected from composite materials, glass ceramics, polymer, metal, metal alloys, or any combination thereof.

10. The implant according to claim 9, wherein the metal or metal alloy is at least partially made of titanium, titanium based alloys, stainless steel, tantalum, zirconium, nickel, tantalum, iridium, nobium, palladium, nickel-titanium, alloys based thereon or any combination thereof.

11. A method of preparing calcium phosphate embedded organic polyelectrolytes compositions; said method comprising *inter alia* the steps of:
    a. adsorbing polyelectrolytes on top of a surface so that at least one polyelectrolyte film is obtained; and,
    b. depositing calcium containing compositions on top of said polyelectrolyte multilayer film, so that at least one of nanometer to micron-sized layer comprising calcium phosphate is formed; so a calcified SAPF is obtained.

12. The method according to claim 9 comprising *inter alia* the steps of:
    a. adsorbing polyelectrolytes on top of a surface so that at least one polyelectrolyte film is obtained;
    b. washing the obtained film in the manner that residual polyelectrolytes are removed;
    c. depositing nano-sized to micron-sized particles of bioactive glass, amorphous calcium phosphate (ACP) and/or crystalline calcium phosphate particles on top of the obtained polyelectrolyte multilayer film, so that at least one layer comprising calcium-containing bioactive inorganic material is obtained; and,
    d. washing the obtained calcified film in the manner that residual calcium containing solution is removed;

wherein an SAPF which is at least partially covered by a single inorganic layer is obtained.

13. The method according to claim 9 comprising *inter alia* the steps of:
    a. adsorbing polyelectrolytes on top of a surface so that at least one polyelectrolyte film is obtained;
    b. washing the obtained film in the manner that residual polyelectrolytes are removed;
    c. depositing nanosized to micron-sized particles of bioactive glass, amorphous calcium phosphate (ACP) and/or crystalline calcium phosphate particles on top of said film, so that at least one of layer comprising calcium-containing, bioactive inorganic material is obtained;
    d. washing the obtained calcified film in the manner that residual calcium containing solution is removed; and,
    e. adsorbing polyelectrolytes on top of said calcium phosphate layer;

    wherein said sequence of steps is repeated in the manner that calcified SAPF is obtained.

14. The method according to claim 9 comprising *inter alia* the steps of:
    a. adsorbing polyelectrolytes on top of a surface so that at least one film is obtained;
    b. washing the obtained film in the manner that residual polyelectrolytes are removed;
    c. depositing nanosized to micron-sized particles of bioactive glass and/or ACP on top of said SAPF, so that at least one film comprising calcium-containing, bioactive inorganic material is formed;
    d. washing the obtained film in the manner that residual calcium containing solution is removed; and,
    e. immersing the material into a calcifying solution in the manner that the growth of crystalline calcium phosphate is induced and sustained.

15. The method according to claim 9 comprising *inter alia* the steps of:

a. adsorbing polyelectrolytes on top of a surface so that at least one film is obtained;

b. washing the obtained film in the manner that residual polyelectrolytes are removed;

c. depositing nano-sized to micron-sized particles of bioactive glass, and/or ACP on top of said SAPF, so that at least one of film comprising calcium-containing bioactive inorganic material is formed;

d. washing the obtained calcified film in the manner that residual calcium containing solution is removed;

e. adsorbing polyelectrolytes on top of said calcium phosphate layer; wherein said sequence of steps is repeated in the manner that calcified SAPF is obtained; and,

f. immersing the obtained material into a calcifying solution in the manner that *in situ* growth of calcium phosphate crystals is induced and sustained within the calcified SAPF.

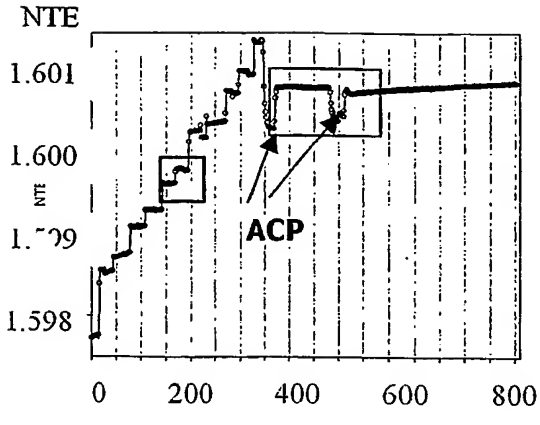
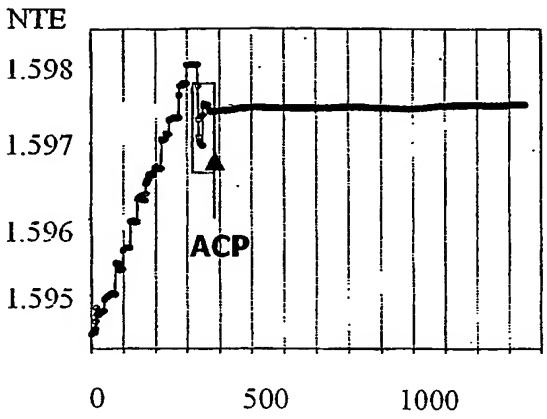


Fig. 1A time / min



time / min Fig. 1B

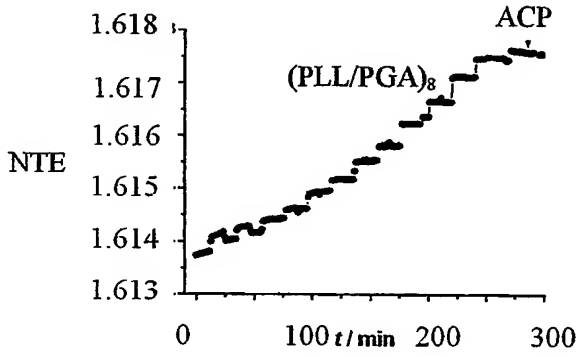
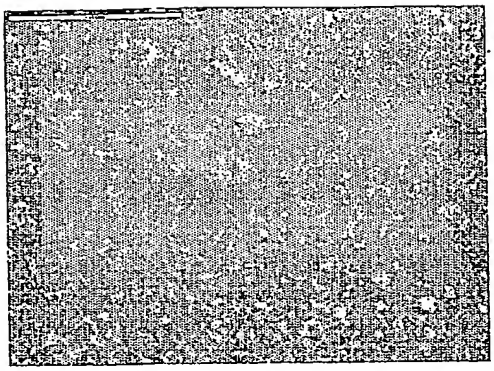
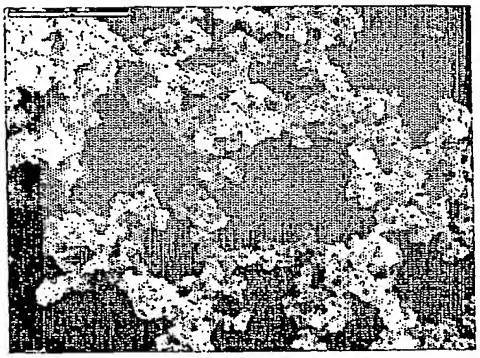


Fig. 1C



Bar 100 μm

Fig. 2A



Bar 5 μm

Fig. 2B

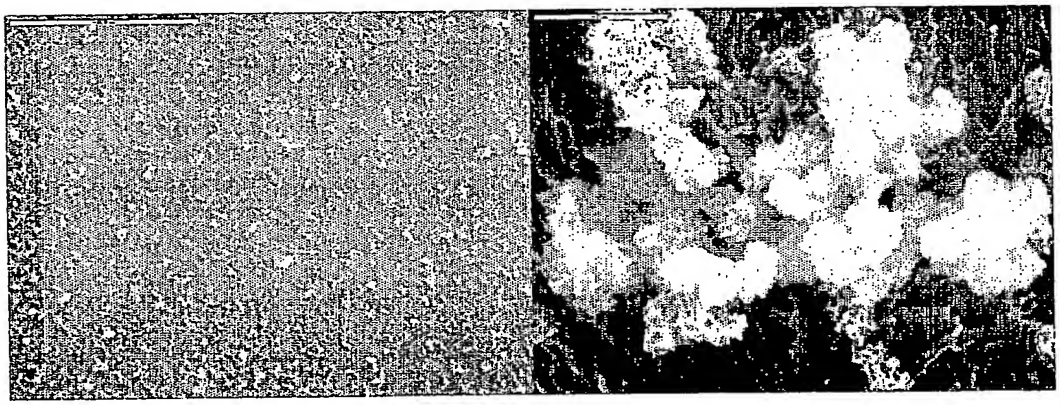


Fig. 3A

Fig. 3B

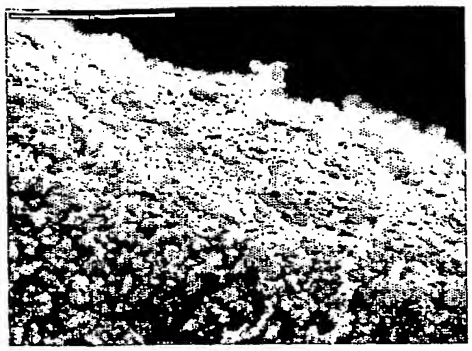
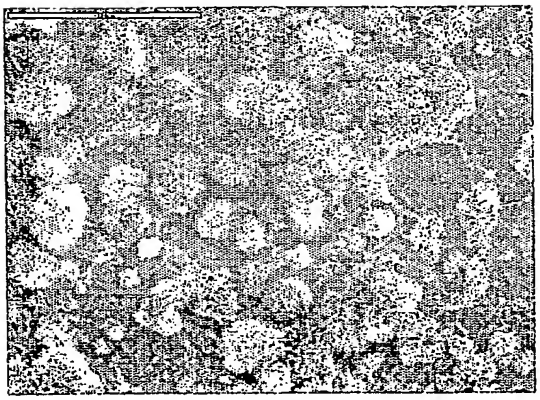


Fig. 4A

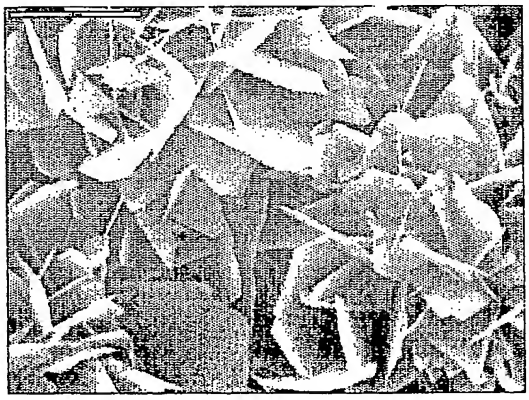
Bar 5 μm

Fig. 4B



Bar 100 μm

Fig. 5A



Bar 5 μm

Fig. 5B

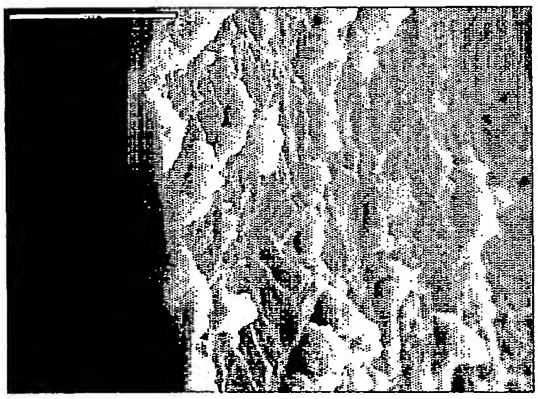
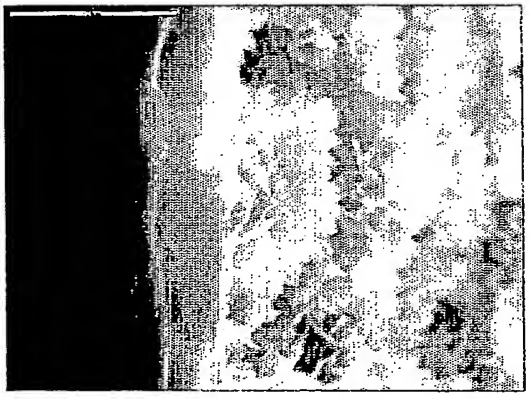


Fig. 6A



Bar 20 μm

Fig. 6B

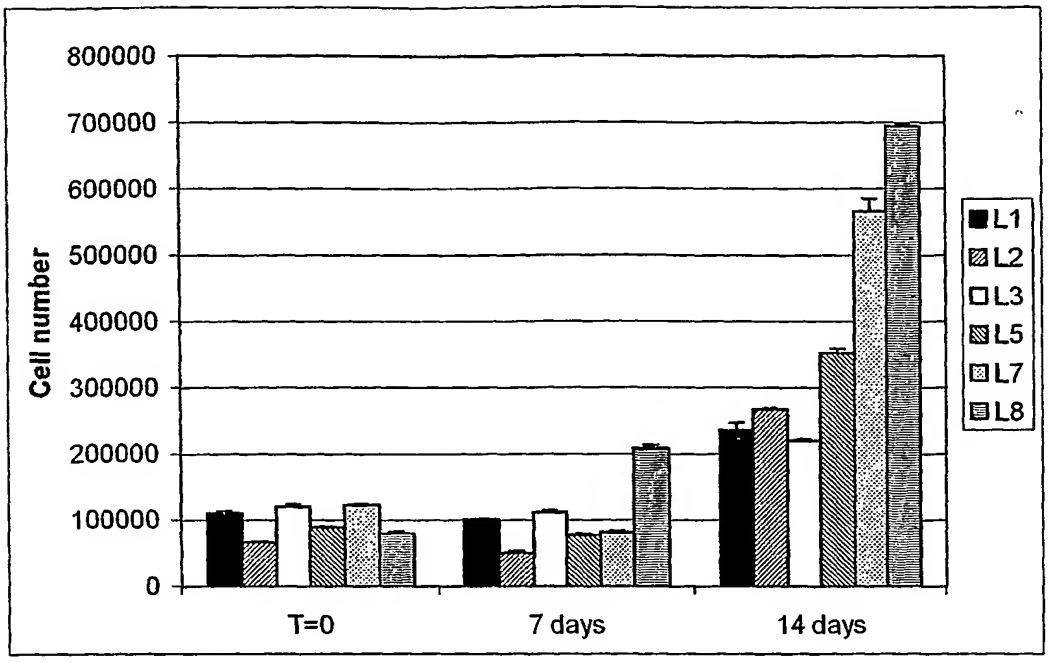


FIGURE 8

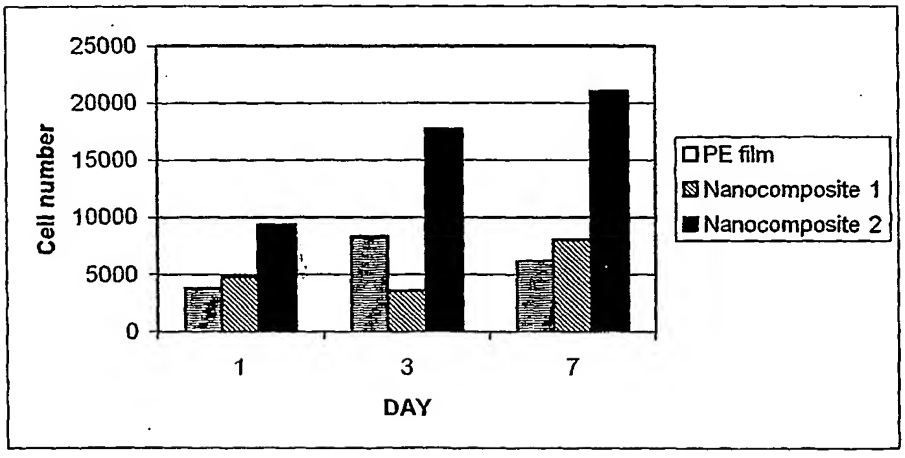


FIGURE 9

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 03/00975

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L27/34 A61L27/32 A61L27/46 B05D1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L B05D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, EMBASE, COMPENDEX, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 166 804 A (MERCK PATENT GMBH) 2 January 2002 (2002-01-02) cited in the application page 3, line 19 - line 32 page 4, line 3 - line 11 ---	1-15
X	HWANG J J ET AL: "ORGANOAPATITE GROWTH ON AN ORTHOPEDIC ALLOY SURFACE" JOURNAL OF BIOMEDICAL MATERIALS RESEARCH, WILEY., NEW YORK, NY, US, vol. 47, no. 4, 15 December 1999 (1999-12-15), pages 504-515, XP009011983 ISSN: 0021-9304 page 505 -page 506; figure 2 --- -/--	1-15

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 March 2004	06/04/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Staber, B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 03/00975

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCHAAF P ET AL: "Films bioactifs destines au recouvrement de biomateriaux et ciblant une regeneration tissulaire: De nouvelles voies originales?" PATHOLOGIE BIOLOGIE, vol. 50, no. 3, 2002, pages 189-193, XP002273840 ISSN: 0369-8114 page 192, paragraph 2; figure 2B	1-10
X	WO 01 78906 A (SPILLMAN WILLIAM B JR ;VIRGINIA TECH INTELL PROP (US); CLAUS RICHA) 25 October 2001 (2001-10-25) page 5, line 12-18 page 6, line 7 - line 13 page 12, line 8 - line 18 page 12, line 19 - line 29	1,2,6-10
X	EP 0 972 563 A (MAX PLANCK GESELLSCHAFT) 19 January 2000 (2000-01-19) page 4, line 10 - line 13 page 4, line 39-50	1,2,6-10
X	ROSIDIAN A ET AL: "IONIC SELF-ASSEMBLY OF ULTRAHARD ZRO2/POLYMER NANOCOMPOSITE THIN FILMS" ADVANCED MATERIALS, VCH VERLAGSGESELLSCHAFT, WEINHEIM, DE, vol. 10, no. 14, 1 October 1998 (1998-10-01), pages 1087-1091, XP000781868 ISSN: 0935-9648 figure 2	1,2,6
A	FUREDI-MILHOFER H ET AL: "Interactions between polyelectrolytes and sparingly soluble salts" PROGRESS IN CRYSTAL GROWTH AND CHARACTERIZATION OF MATERIALS, ELSEVIER PUBLISHING, BARKING, GB, vol. 32, no. 1, 1996, pages 45-74, XP004048531 ISSN: 0960-8974 page 47 page 53 page 59 -page 60; figure 10	1-15

-/--

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 03/00975

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MICKIEWICZ RAFAL A ET AL: "Polymer-calcium phosphate cement composites for bone substitutes" JOURNAL OF BIOMEDICAL MATERIALS RESEARCH, vol. 61, no. 4, 15 September 2002 (2002-09-15), pages 581-592, XP002273841 ISSN: 0021-9304 page 582, paragraph 5	1-15

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 03/00975

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 1166804 A	02-01-2002	DE 10029520 A1	17-01-2002
		AT 246525 T	15-08-2003
		CA 2351009 A1	21-12-2001
		DE 50100456 D1	11-09-2003
		EP 1166804 A2	02-01-2002
		JP 2002035107 A	05-02-2002
		US 2004033249 A1	19-02-2004
		US 2002018798 A1	14-02-2002
WO 0178906 A	25-10-2001	AU 5344201 A	30-10-2001
		CA 2405242 A1	25-10-2001
		EP 1280613 A1	05-02-2003
		JP 2003530926 T	21-10-2003
		WO 0178906 A1	25-10-2001
		US 2002037383 A1	28-03-2002
EP 0972563 A	19-01-2000	EP 0972563 A1	19-01-2000
		AT 228883 T	15-12-2002
		DE 29924358 U1	02-01-2003
		DE 69904307 D1	16-01-2003
		DE 69904307 T2	04-09-2003
		WO 9947253 A1	23-09-1999
		WO 9947252 A2	23-09-1999
		WO 0003797 A1	27-01-2000
		EP 1064087 A2	03-01-2001
		EP 1064088 A1	03-01-2001
		EP 1098696 A1	16-05-2001
		JP 2002506719 T	05-03-2002
		JP 2003522621 T	29-07-2003
		JP 2002520151 T	09-07-2002
		US 2003219384 A1	27-11-2003
		US 6479146 B1	12-11-2002
		US 6699501 B1	02-03-2004